# Design of soluble epoxide hydrolase inhibitors as drug leads

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Epoxyeicosatrienoic acids (EETs) are generated from arachidonic acid by epoxygenases of the cytochrome P450 superfamily. In humans, EETs promote vasodilation and angiogenesis, and act to inhibit systemic anti-inflammatory response (1). The enzyme soluble epoxide hydrolase (sEH) breaks down EETs into dihydroxyeicosatrienoic acids (DHETs). A drug-like inhibitor of sEH could maximize the concentration of EETs in the blood and thus have utility for the treatment of cardiovascular and inflammatory diseases (2).

In this project, a number of putative sEH inhibitors were designed. Work was based on previous drug design efforts as well as on the three-dimensional structure of the human enzyme (3). sEH crystal structures exhibit two domains with distinct activities—the C-terminal domain catalyzes the epoxide hydrolysis reaction for which the enzyme is named, whereas the N-terminal domain exhibits phosphatase activity that is reportedly independent—at least kinetically—of the epoxide reaction (4). Three amino acids in the hydrolase active site (Asp333, Tyr381, and Tyr465) that are essential to catalysis also participate in hydrogen-bonding interactions with inhibitors. The hydrolase active site lies in a large, 25 Å-long hydrophobic cavity in the C-terminal domain. Van der Waals interactions with a number of nonpolar residues contribute to hydrolase inhibitor binding. Inhibitor design targeted the hydrolase active site; however, a known inhibitor of the phosphatase active site was linked to putative hydrolase inhibitors to create bi-substrate inhibitors, which are expected to bind sEH with considerable affinity due to entropic effects (5).

## Introduction

Epoxyeicosatrienoic acids (EETs) are formed in mammalian tissues by epoxygenases of the cytochrome P450 (CYP) superfamily that use arachidonic acid as their substrates. Various EET regioisomers (including the 5,6-, 8,9-, 11,12-, and 14,15-EETs generated by CYP) are known to have biological activities that render them potentially beneficial for human health, although their mechanism of action is poorly understood (Figure 1). Increasing the biological concentration of EETs is emerging as a strategy for the treatment of cardiovascular disease and inflammatory disorders (2).

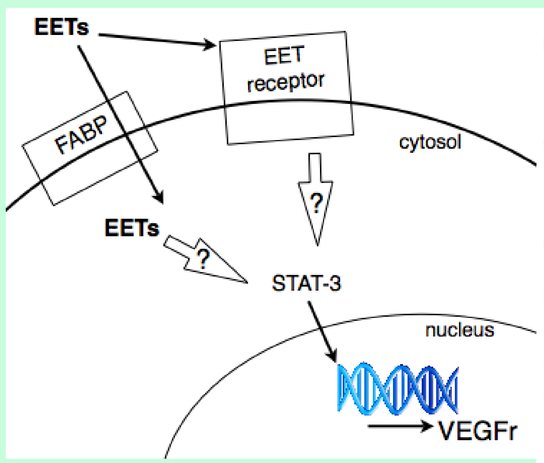


Figure 1. Extracellular EETs may, by alternate hypotheses, bind to a selective EET receptor (6) or to a fatty-acid binding protein (FABP). Then, either indirectly or directly, EETs may initiate a signal transduction cascade and/or modulate gene expression (7). EETs may ultimately activate STAT-3, which induces expression of the VEGF receptor; this may explain EETs' pro-angiogenic activity (8).

The enzyme soluble epoxide hydrolase (sEH) degrades EETs into dihydroxyeicosatrienoic acids (DHETs) and to a large degree controls EET levels (9). sEH has two distinct structural domains that catalyze separate reactions, a carboxy-terminal epoxide hydrolase domain and a less-well-characterized amino-terminal lipid-phosphate phosphatase domain. Active, human sEH is a homodimer with a domain-swapped architecture. It stabilizes the substrate with hydrogen bond donors Tyr381 and Tyr465 at the epoxide oxygen. Asp333 initiates the hydrolysis reaction by nucleophilic attack (10, 11).

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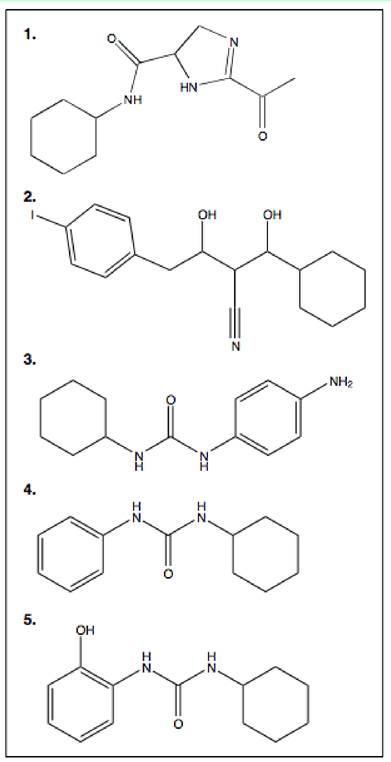
It has been proposed that sEH inhibition could increase physiological concentrations of EETs by extending the half-lives of the compounds (9). A number of drug leads have been designed with the goal of sEH inhibition. Successful inhibitor design studies have identified a primary pharmacophore region that participates in key hydrogen bonding interactions with the catalytic core of the hydrolase active site (2). This pharmacophore is best represented by 1,3-disubstituted ureas, carbamates, and amides, all of which are transition-state analog inhibitors of the enzyme (12).

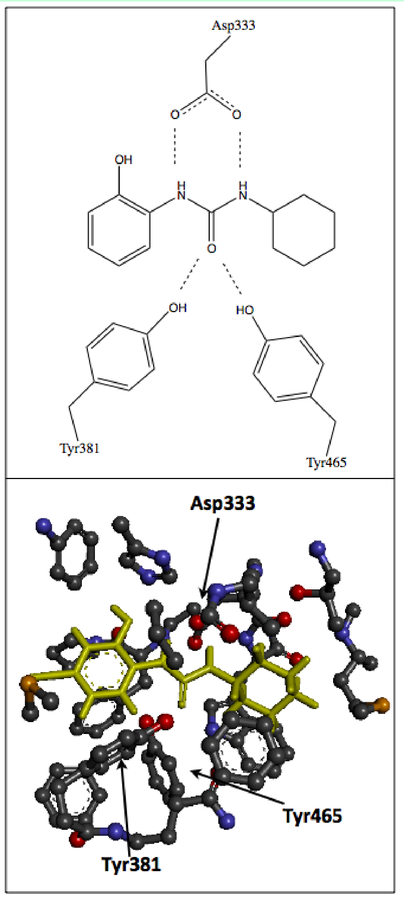
## Methods

The Imig laboratory has helped develop EET analogs that mimic the actions of endogenous EETs, yet resist metabolism by sEH. The ability of the analogs to dilate the afferent arteriole in male rats was evaluated with renal vascular experiments. Blood pressure and heart rate were monitored using telemetry transmitters. Their work established 11,12-ether-EET-8-ZE as a promising lead and provided the first evidence that EET analogs have potential for the treatment of cardiovascular disease (14).

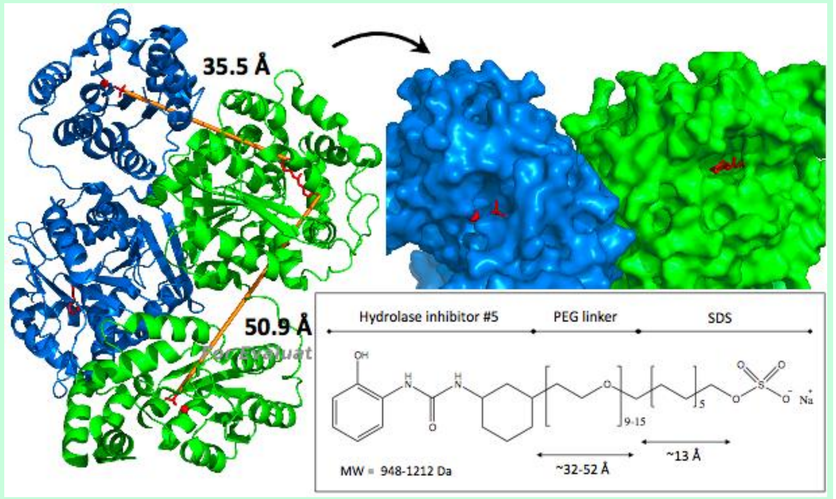
In this project, the X-ray crystal structure of sEH in complex with a urea-based inhibitor (PDB ID 1VJ5) was studied with protein visualization software (Jmol, Swiss-PDB Viewer). Interactions between the protein and the inhibitor were investigated. Based on these findings, other inhibitor-like molecules were built in Spartan and minimized according to semi-empirical energy calculations. These molecules were imported into Discovery Studio Visualizer and modeled into the active site of sEH. Those with appropriate dimensions and hydrogen bond distances to catalytic core residues are pictured below. One molecule was chosen as the preferred candidate for inhibition. Bi-substrate inhibitors were designed by linking this molecule to sodium dodecyl sulfate, a known inhibitor of the phosphatase active site (13). The length of the PEG linker region was estimated using measurement analysis tools in PyMOL.

## Results




Left: Putative hydrolase inhibitors 1-5. Top right: Molecule 5, predicted hydrogen-bond interactions in sEH C-terminal active site. Bottom right: Molecule 5 modeled into 1VJ5 C-terminal active site.




Left: sEH catalytic unit (1VJ5, PyMOL). Distances (orange rods) measured between ligands (red). Distance between ligands on subunit A (green) is ~50.9 Å. Distance between C-terminal ligand in A and N-terminal ligand in subunit B (blue) is ~35.5 Å. Ligand in C-terminal site is N-cyclohexyl-N'-(4-iodophenyl)urea. Ligand in N-terminal site is $PO_4^{3-}$ coordinated by $Mg^{2+}$. Top right: View of N-terminal (left) and C-terminal (right) active sites across the subunit interface. Protein displayed as a van der Waals surface. Distance measurements across this surface were used to estimate the length of PEG linkage needed. Bottom right: Putative bi-substrate sEH inhibitor.

## What's Next?

The next step for the molecules designed in this study is to determine how they interact with sEH. Kinetic assays *in vitro* would help determine the dissociation constant for each enzyme-inhibitor complex, as well as the mode of inhibition for each molecule. While hydrolase domain inhibitors are generally understood to be competitive inhibitors, the mode of inhibition for a bi-substrate sEH inhibitor has not yet been investigated.

Subsequently, the drug-like nature of each inhibitor would need to be assessed via ADMET testing, either *in silico* or *in vivo*. *In silico* methods might involve docking to hepatic enzymes that frequently degrade drug leads or using software tools such as TOPKAT (Accelyris) to assess toxicity and predict therapeutic dosage.

## Summary

A small library of putative sEH inhibitors was designed using the three-dimensional structure of the human enzyme, as well as knowledge generated by previous sEH inhibitor design projects. These molecules, should they be potent and specific sEH inhibitors, could have utility for the treatment of cardiovascular and inflammatory diseases.

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